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## SPECIAL ISSUE ARTICLE

# Changes in host ant communities of Alcon Blue butterflies in abandoned mountain hay meadows

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**Abstract.** 1. Land use intensification is a general threat to biodiversity, but many species depend on low-intensity agricultural ecosystems. One example is European mountain meadow ecosystems, traditionally managed by hay harvesting or livestock grazing. Abandoning management often causes population declines, local extinctions and biotic homogenisation in these meadows.

2. We studied changes in the *Myrmica* host ant communities of the xerophilic form of the ant-parasitic Alcon Blue butterfly (*Maculinea alcon*) in four hay meadows in the Bükk mountains of Hungary between 2000–2007 and 2018. Abandonment started in this region in the 1970s, accelerated in the 1980s, and culminated in the 1990s.

3. We found that the *Myrmica* ant community has changed substantially in less than two decades. Diversity of the ant community always decreased, and species composition became more homogeneous at two sites. Habitat suitability for *Maculinea* butterflies decreased at three sites and increased at only one site, where management was restarted 20 years after abandonment.

4. The number of *M. alcon* caterpillars and pupae, the rate of infestation of ant nests and the mean number of caterpillars and pupae per ant nest decreased between the two periods, whereas host ant specificity did not differ from random in either period.

5. We conclude that the unfavourable changes in the host ant community due to abandonment have negative consequences for the persistence of *Maculinea* populations. Our study highlights the need for detailed monitoring, and the maintenance of low-intensity management by mowing or grazing to avoid the decline of biodiversity dependent on low-intensity agriculture.

**Key words.** Carpathian Basin, management, multiple host ant use, myrmecophily, *Phengaris*, social parasite, succession.

## Introduction

Land use change is one of the major drivers of biodiversity loss in Europe (Rounsevell *et al.*, 2018). In most of Europe, intensification of land use, for example, increased inputs of chemicals in agriculture, is associated with negative impacts on the biodiversity of natural habitats. However, many species and habitats have adapted to long-term low-intensity farming, and farmland biodiversity has also declined considerably in the last few decades

(Kleijn *et al.*, 2009). Mountain hay meadows and pastures are prime examples of human-maintained habitat types in landscapes dominated by forests. These open habitats host unique plant and animal species that are characteristic of both lowland steppes and mountain meadows or grasslands, leading to the formation of highly diverse communities packed with species of biogeographic and conservation importance. For example, mountain hay meadows in Transylvania, Romania hold the world record for plant species richness in 0.1 m<sup>2</sup> and 10 m<sup>2</sup> areas (Wilson *et al.*, 2012). These meadows have been maintained for centuries by regular human use, for example, for harvesting hay or livestock grazing, which effectively prevented the overgrowth of these open habitats by woody plants such as shrubs and trees

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(Vera, 2000). The cessation of traditional land use often results in succession starting with the appearance of woody plants, leading to scrub, then patchy woodland and eventually to closed forest (Baur *et al.*, 2006). In central and eastern Europe, the number of grazing livestock started to decrease in the 1970s and 1980s and fell drastically in the 1990s due to socioeconomic changes and changes in agricultural practices (Prokopová *et al.*, 2018), leading to large-scale abandonment of mountain meadows. As a result, the vegetation and invertebrate fauna of mountain hay meadows have changed considerably since the 1990s (Baur *et al.*, 2006). The maintenance of the biodiversity of these unique, human-maintained habitat types thus requires active management to halt succession and to keep them in their high-diversity state (Stoate *et al.*, 2009).

In many parts of Europe, *Maculinea* (= *Phengaris*) butterflies (Lepidoptera: Lycaenidae) are flagship species due to their complex life cycle and sensitivity to the status of their habitats (Settele *et al.*, 2005). Mountain meadows and semi-dry grasslands in central Europe are often inhabited by the xerophilous form of the Alcon Blue butterfly (*Maculinea alcon* [Denis & Schiffermüller], 1775), which we will refer to as *M. alcon X* (Tartally *et al.*, 2019). This butterfly has an unusual life cycle in which the caterpillars start their development by feeding on the developing seeds of gentian plants. In the final instar, the caterpillars are taken into their nests by *Myrmica* (Hymenoptera: Formicidae) ants, and are raised together with the host brood (Thomas *et al.*, 1989) for 1 or 2 years (Thomas *et al.*, 1998a). The caterpillars mimic the odour (Schönrogge *et al.*, 2004; Thomas *et al.*, 2013) and the sound (Barbero *et al.*, 2009) of the ants and are mostly fed by the *Myrmica* workers by trophallaxis (Thomas & Elmes, 1998), although they also prey directly on the ants' brood (Tartally, 2004). A multitude of studies suggest that different Alcon Blue populations have adapted to different *Myrmica* species as their local hosts (Tartally *et al.*, 2019). There are both multiple-host-ant using populations, typically in central Europe, and more host-specific populations, typically in western Europe (Tartally *et al.*, 2019). The hygrophilic form of *M. alcon* (see 'Study sites and populations' below) uses mainly *M. rubra* and *M. ruginodis* in the north-west of Europe, and mainly *M. scabrinodis* and its close relatives in the remainder of Europe. *M. alcon X* generally exploits *M. sabuleti* or *M. schencki* and has a more diverse host ant usage in central Europe than in the western or northern periphery of its range (Tartally *et al.*, 2019). The *M. alcon* form *rebeli* has been recorded only from *M. sulcinodis* (Tartally *et al.*, 2014). Not

all the available *Myrmica* species are suitable hosts of a given *M. alcon* population (Thomas *et al.*, 1989). Some *Myrmica* species, for example, usually kill the caterpillars after adoption, especially under food stress (Elmes *et al.*, 2004; Thomas *et al.*, 2005). This life cycle makes *Maculinea* butterflies highly sensitive to changes in the local *Myrmica* community. The *Myrmica* communities in turn are sensitive to changes in their microhabitats (Elmes *et al.*, 1998). Therefore, successional changes and/or inappropriate management of a site can cause the decline of the local host ant species, which in turn can result in the local extinction of the *Maculinea* population (Thomas, 1980; Thomas *et al.*, 2009). For these reasons, *Maculinea* butterflies are widely recognised as indicator and umbrella species of their habitats (Settele *et al.*, 2005), and are protected in most countries of Europe (Munguira & Martín, 1999).

Understanding the links between land use or management and *Myrmica* ant communities is thus of fundamental importance in the conservation of species-rich mountain hay meadows and pastures, as well as the persistence of *Maculinea* butterflies. In addition, study of the host ant specificity of *Maculinea* butterflies also has evolutionary relevance. Following the pioneering work of Thomas *et al.* (1989), we have obtained extensive information on the host ant use of *Maculinea* butterflies in different regions of Europe (Tartally *et al.*, 2019), largely as a result of the MacMan collaborative research programme (Settele *et al.*, 2002). These insights (Tartally *et al.*, 2019) aptly demonstrate the spatial dimension of the geographic mosaic of coevolution hypothesis (Thompson, 1999) in the *Maculinea*–*Myrmica* coevolved system. However, to our knowledge, the temporal dimension, that is, the dynamics of this system in time has not yet been sufficiently addressed.

The aim of this study was to evaluate whether and how changes in land use and management influence *Myrmica* ant communities and their infestation by *M. alcon X* caterpillars and pupae in four meadows in the Bükk-mountains of northern Hungary. Because many of the former mountain hay meadows and pastures have already disappeared due to land abandonment, lack of management and shrub encroachment, it was not possible to obtain an adequate sample size to systematically examine different management histories. Instead, we use the four study sites as case studies of different management histories. Traditional land use was abandoned at different times at these four sites and at least one 10-year period of no management occurred on each of them since the 1970s (see Materials and Methods section, Table 1). A previous publication (Tartally *et al.*, 2008)

**Table 1.** History of land use or management of the study sites based on personal observations of ZV and AT, and consultation with local farmers and with experts from Bükk National Park. Dashes indicate no management.

Period	Site			
	Bükkszentkereszt	Kecskeláb-rét	Lófő-tisztás	Nagy-mező
1970–1979	Mowed, cattle	Mowed	Cattle, mowed	Mowed
1980–1989	Mowed, cattle	-	Cattle, mowed	Horses
1990–1999	Mowed, cattle	-	-	Horses
2000–2009	-	-	-	Horses
2010–2018	-	Mowed once ca. 2010	Mowed, cattle	Burned in 2012

reported on our studies of *Myrmica* communities and *Maculinea* host ant use carried out between 2000 and 2007 at these four sites and several others. In 2018, we resampled these four sites and evaluated the impact of habitat management and vegetation succession on host ant communities, *Maculinea* numbers and infestation rates, and host ant specificity. We compare these results to those of our previous study (Tartally *et al.*, 2008) and develop recommendations for habitat management to increase the chance of persistence of *Maculinea* butterfly populations.

## Materials and methods

### Study sites and populations

Current literature (Tartally *et al.*, 2019) distinguishes three forms of *M. alcon*, the hygrophilic (*M. alcon H*), the xerophilic (*M. alcon X*) and the Alpine (*M. alcon* form *rebeli*). For a long period *M. alcon X* was widely referred to as '*M. rebeli*'; however, this assertion has been shown to be misleading (Habeler, 2008; Kudrna & Fric, 2013; Tartally *et al.*, 2014; Koubínová *et al.*, 2017; Bereczki *et al.*, 2018). In this study, we focused exclusively on habitats of *M. alcon X*. This form occurs in drier or mesic areas, often at intermediate and higher elevations, and mostly uses *Gentiana cruciata* L. as a host plant for egg-laying and early larval development.

In all four sites, we selected patches with high *G. cruciata* density for detailed examination. Patches were selected haphazardly from across the site, but were chosen so as to also leave other nearby patches with high *G. cruciata* density undisturbed. When other host plants were found within a patch (i.e., *Gentiana pneumonanthe* L. and *Gentianella austriaca* (A. & J. Kern) Holub, see below), that patch was not sampled.

**Bükkszentkereszt** (48°04'N, 20°38'E, 563 m above sea level, a.s.l.). This small meadow, surrounded by sparsely populated residential areas was regularly mowed and grazed by cattle from at least 1970. Combined land use by early summer mowing and late summer cattle-grazing continued longest at this site, and the meadow was abandoned in the late 1990s. The vegetation of the site had very few woody plants before abandonment, and a substantial part of the site has been overgrown by shrubs since. The area of the selected patches at this site was 3161 m<sup>2</sup> in total.

**Kecskeláb-rét** (48°05'N, 20°31'E, 751 m a.s.l.). This large meadow was regularly mowed at least from 1960, but this management ceased at the end of the 1970s. The meadow was subsequently mowed a single time around 2010 by Bükk National Park, making this the site with the longest period without management. This site still had rather open vegetation in 2018, presumably due to the grazing of wild ungulates. The area of the selected patches at this site was 7373 m<sup>2</sup>.

**Lófé-tisztás** (48°04'N, 20°39'E, 656 m a.s.l.). This large meadow was regularly mown by farmers for hay and was also regularly grazed since at least 1970. The meadow was abandoned in the early 1990s. Management was re-started in 2010 by regular annual mowing and low-intensity cattle-grazing.

*Gentianella austriaca* is an additional host plant of the local *M. alcon X* population. The area of the selected patches at this site was 6189 m<sup>2</sup>.

**Nagy-mező** (48°04'N, 20°30'E, 783 m a.s.l.). This large meadow was regularly mowed at least from 1960. At the end of the 1970s, management by horse-grazing was started at the site. Horse-grazing was terminated around 2010 and the site has not been managed since, making this site the most recently abandoned. *Gentiana pneumonanthe* is an additional host plant of the local *M. alcon X* population. This population does not have clearly separated flying periods associated with the different flowering periods of the two host plants (János Tóth & Tamás Korompai, pers. comm.), unlike the Romanian site Răscruți. At that site there is strong genetic differentiation between the xerophilous form (*M. alcon X*) using *Gentiana cruciata* and the hygrophilous form (*M. alcon H*) developing on the *G. pneumonanthe* and the plants (Tartally *et al.*, 2016). The area of the selected patches at this site was 5330 m<sup>2</sup>.

### Sampling

To count *Maculinea alcon X*, we searched all *Myrmica* nests less than 2 m distant from every stalk of *G. cruciata* in each selected patch for caterpillars, pupae and pupal exuviae. This area corresponds to the approximate space where foragers from *Myrmica* nests are likely to encounter caterpillars of *M. alcon X* emerging from the host plants (Elmes *et al.*, 1998). Ant nests were examined in the early summer, immediately before the flight period of each population, to ensure that any *M. alcon X* caterpillars and pupae found had survived the winter, most likely as an integrated part of the colony investigated (Thomas *et al.*, 2005). As no 2-year developing larvae (Schönrogge *et al.*, 2000; Witek *et al.*, 2006) are known from Hungary (Tartally, 2005), and all larvae found in this study were of similar size, we did not exclude any larvae from the analyses.

We recorded *Myrmica* nests, opened them carefully and counted the number of *M. alcon X* caterpillars and pupae. Five to 10 *Myrmica* workers per colony were collected and stored in 70% ethanol. We identified worker ants to species in the laboratory [according to the keys of Seifert (1988), Radchenko and Elmes (2010), Czechowski *et al.* (2012)], using a Leica MZ125 microscope (magnification 10–160×).

Sampling was carried out every year on each site during the 2000–2007 period, but different patches were sampled each year, and the data of the different patches and years were pooled for analysis. All the patches investigated during 2000–2007 were re-sampled in 2018, so the exact areas surveyed were comparable between the two periods.

### Data analysis

The *Myrmica* communities (proportion of nests of different *Myrmica* species) were compared between the two collection periods at each site using a Fisher's exact test. We used the R 'stats' package for calculating Fisher's exact tests for

contingency tables with two columns (periods) and  $N$  rows, where  $N$  was the number of species. We used Shannon's diversity index calculated using PAST v. 3.20 (Hammer *et al.*, 2001) to characterise *Myrmica* species diversity in the two periods. We visualised similarity of and changes in *Myrmica* community with multidimensional scaling (MDS) ordination in JMP 14.3 (SAS institute, 2019) using a matrix of pairwise Bray–Curtis dissimilarities between *Myrmica* communities calculated using PAST and based on the relative abundance of each *Myrmica* species.

We characterised the suitability of the ant community for the *Maculinea* population at a site using the following *Myrmica* community suitability index (MCSI):

$$\text{MCSI} = \frac{N_{\text{Maculinea caterpillars and pupae}}}{N_{\text{Myrmica nests}}}$$

where  $N_{\text{Maculinea caterpillars and pupae}}$  was the number of all *Maculinea* caterpillars and pupae recorded in all the examined *Myrmica* nests at a site in a study period, and  $N_{\text{Myrmica nests}}$  was the number of the examined *Myrmica* nests. This index gives the average number of *M.alcon X* caterpillars and pupae per *Myrmica* nest, and as such we assume that the higher the value of the index, the more suitable is the *Myrmica* community for the *Maculinea* population. It is important to note that this simple index does not take into account any host ant specificity, so is not generally applicable to all *Maculinea* sites. Our previous study, however, found little or no host ant specificity in the studied populations (Tartally *et al.*, 2008).

For number of caterpillars and pupae and infestation rates, although the distribution of most variables departed from normality, we present means  $\pm$  SDs for comparability with previous studies. We used the median test to statistically compare medians of distributions.

We calculated host ant specificity, that is, the deviation from a random occurrence of *Maculinea* caterpillars and pupae in nests of different *Myrmica* species in two ways: (i) using Fisher's exact tests of heterogeneity of whether nests of different *Myrmica* species are infected by *M.alcon X* or not, and (ii) by estimating the likelihood that the numbers of *M.alcon X* caterpillars and pupae would be distributed as heterogeneously as observed between nests of different *Myrmica* species by constrained randomisation of ant nests between species using the software MacSamp (Tartally *et al.*, 2008). The latter takes into account the number of caterpillars and pupae found within nests as well as whether nests are infested or not.

Distinguishing between the closely related *M.sabuleti* Meiner, 1861 and *M.lonae* Finzi, 1926 can be problematic (Seifert, 2000; Czechowski *et al.*, 2012). These two similar ants are discussed here as separate species, but their relationship needs clarification (Radchenko & Elmes, 2010; Seifert, 2018). Sharing of mitochondrial haplotypes suggests that they may not be separate species (Ebsen *et al.*, 2019), as does their similarity in cuticular hydrocarbons (Guillem, 2014). Because of this taxonomic uncertainty and the difficulty of identification of these two ants, we repeated all analyses assuming that *M.lonae* nests identified in 2000–2007 were actually *M.sabuleti* nests. We note differences only if the results of this repeated analysis differed from the original analysis.

## Results

### Ant species and community composition

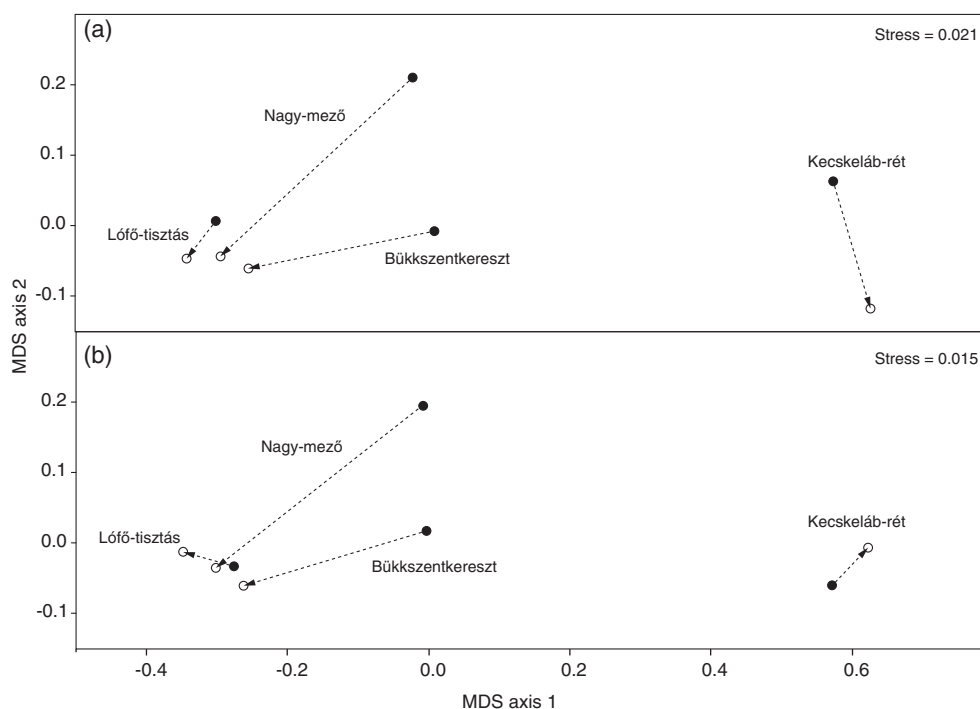
In the two periods combined, we examined 625 nests of six *Myrmica* species (*M.scabrinodis* Nylander, 1846; *M.sabuleti*;

**Table 2.** The number of nests found, the number of nests infested with *M.alcon X* caterpillars and pupae and the total number of caterpillars and pupae in nests of the different *Myrmica* species found at each site in each period.

Site	<i>Myrmica</i> species	N° nests		N° infested nests		N° caterpillars and pupae	
		2000–2007	2018	2000–2007	2018	2000–2007	2018
Bükkszentkereszt	<i>ruginodis</i>	0	1				
	<i>sabuleti</i>	30	7	3		4	
	<i>scabrinodis</i>	55	86	2	1	24	1
	<i>schlencki</i>	0	6		1		1
Kecskeláb-rét	<i>lonae</i>	3	0	1		5	
	<i>sabuleti</i>	21	47	1	2	6	3
	<i>scabrinodis</i>	2	0				
	<i>schlencki</i>	0	3				
Lófő-tisztás	<i>lonae</i>	8	0				
	<i>ruginodis</i>	2	2				
	<i>sabuleti</i>	5	0	1		5	
	<i>scabrinodis</i>	149	49	7	5	82	43
Nagy-mező	<i>schlencki</i>	4	2	1	1	15	2
	<i>lobicornis</i>	2	0				
	<i>lonae</i>	4	0				
	<i>ruginodis</i>	10	1				
	<i>sabuleti</i>	22	3	2		6	
	<i>scabrinodis</i>	47	49				
	<i>schlencki</i>	4	1				

**Table 3.** Indices and probabilities for comparison of sites between survey periods. ‘*P* same’ is the two-tailed probability from Fisher’s exact tests comparing the distribution of ant nests amongst *Myrmica* species between the two periods. *P1* is the two-tailed probability from Fisher’s exact tests of heterogeneity in infestation of host ant nests. *P2* is the probability from randomisation tests of ant nests between species (using MacSamp). MCSI is the *Myrmica* community suitability index at each site in 2000–2007 and 2018. Results are presented separately for analyses treating the *Myrmica lonae* nests found in the first survey as belonging to a distinct species, and combining nests of *M. lonae* and *M. sabuleti*. *P*-values less than 0.05 are marked in bold. See text for further details.

Site				Host specificity				MCSI	
				<i>P1</i>		<i>P2</i>			
	Shannon diversity		<i>P</i> same	2000–2007	2018	2000–2007	2018	2000–2007	2018
2000–2007	2018	2000–2007		2018	2000–2007	2018	2000–2007	2018	
Treating <i>M. lonae</i> as a separate species									
Bükkszentkereszt	0.649	0.531	<b>&lt;0.001</b>	0.340	0.140	0.248	0.063	0.329	0.030
Kecskeláb-rét	0.619	0.227	<b>0.005</b>	0.354	>0.999	0.253	0.558	0.423	0.060
Lófő-tisztás	0.498	0.320	0.172	0.151	0.391	0.214	0.141	0.607	0.849
Nagy-mező	1.292	0.397	<b>&lt;0.001</b>	0.340	–	0.259	–	0.067	0.000
Combining <i>M. lonae</i> with <i>M. sabuleti</i>									
Bükkszentkereszt	0.649	0.531	<b>&lt;0.001</b>	0.340	0.140	0.250	0.063	0.329	0.030
Kecskeláb-rét	0.271	0.227	0.096	>0.999	>0.999	0.576	0.556	0.423	0.060
Lófő-tisztás	0.446	0.320	0.052	0.219	0.391	0.272	0.136	0.607	0.849
Nagy-mező	1.167	0.397	<b>&lt;0.001</b>	0.292	–	0.181	–	0.067	0.000



**Fig. 1.** MDS ordination plot showing changes in *Myrmica* species composition at the four sampling sites between 2000–2007 and 2018. Arrows point from communities in 2000–2007 (solid symbols) to the communities at the same sites in 2018 (open symbols). MDS is based on Bray–Curtis dissimilarities between communities based on relative species abundance. (a) Treating *Myrmica lonae* nests found in the first survey as belonging to a distinct species, and (b) Combining nests of *M. lonae* and *M. sabuleti*.

*M. lonae*; *M. schencki* Viereck, 1903; *M. lobicornis* Nylander, 1846; and *M. ruginodis* Nylander, 1846) at the four sites. We found 368 nests in 2000–2007 and 257 nests in the same patches in 2018 (Table 2), but there was no consistent change in ant nest

density between periods, as more nests were found in the first period at two sites and at the second period in the other two. *Myrmica scabrinodis* was the most abundant species at three sites (Bükkszentkereszt, Lófő-tisztás, Nagy-mező) and *M. sabuleti*

was the most abundant species at the remaining site (Kecskeláb-rét). We found all six *Myrmica* species in 2000–2007, but only four in 2018; the two *Myrmica* species missing in 2018 were *M. lobicornis* and *M. lona*.

When data from all sites were pooled, the Shannon diversity of *Myrmica* species decreased from 0.940 (or 0.828 if *M. lona* individuals are considered *M. sabuleti*) in 2000–2007 to 0.781 in 2018. The distribution of ant nests amongst *Myrmica* species differed significantly in the two periods (Fisher's exact test:  $P < 0.001$ ) but not when *M. lona* individuals were considered *M. sabuleti* ( $P = 0.153$ ).

The distribution of ant nests amongst *Myrmica* species differed between the two periods at three of the four sites (Table 3). When *M. lona* individuals found in 2000–2007 were considered as *M. sabuleti*, this difference remained significant for Nagy-mező ( $P < 0.001$ ) and became marginally non-significant for Kecskeláb-rét ( $P = 0.096$ ) and also for Lófő-tisztás ( $P = 0.052$ ), where these proportions did not differ in the first analysis (Table 3).

The proportion of ant species considered as hosts (Tartally *et al.*, 2008) was 92.1% (or 96.2% if *M. lona* is considered *M. sabuleti*) in 2000–2007 and 98.4% in 2018 (Fisher's exact test between periods:  $P < 0.001$  or  $P = 0.143$  if *M. lona* are *M. sabuleti*). However, values of the MCSI decreased at least one order of magnitude from 2000–2007 to 2018 at three sites and increased by around 40% in Lófő-tisztás (Table 3). When data from all sites were combined, MCSI decreased from 0.399 in 2000–2007 to 0.198 in 2018.

MDS ordination suggested that species composition changed in different ways at the sites between 2000–2007 and 2018 (Fig. 1; Table 2). Communities in Lófő-tisztás and Nagy-mező became more similar in 2018 because the number of species decreased from five to three and six to four respectively, and both communities became dominated by *M. scabrinodis*. Both of these communities converged on that found at Bükkszentkereszt, where *M. scabrinodis* was already dominant in 2000–2007. However, the total number of *Myrmica* species found at this latter site increased from two in 2000–2007 to four in 2018. Finally, the ant community at Kecskeláb-rét was dominated by *M. sabuleti* in both periods and changed in parallel with the other three sites because two of the species found in 2000–2007 were not found in 2018 and one species not found in 2000–2007 appeared at the site in 2018. These patterns of change were largely consistent regardless of whether *M. lona* was treated as a separate species or combined with *M. sabuleti* (Fig. 1).

#### Larval numbers and infestation rates

In 2000–2007, we found 147 *M. alcon* X caterpillars and pupae in 18 of the 368 ant nests studied, whilst in 2018, we found 51 caterpillars and pupae in 11 out of the 257 nests. The decrease in the total number of *M. alcon* X caterpillars and pupae (ca. 65%) was much larger than the decrease in the number of nests found (30%), indicating that the decrease in the number of caterpillars and pupae could not be attributed only to differences in sampling effort.

The rate of infestation decreased slightly from 4.9% of all nests in 2000–2007 ( $n = 368$ ) to 4.3% of all nests in 2018

( $n = 257$ ). The mean number of *M. alcon* X caterpillars and pupae per nest decreased from  $0.399 \pm (\text{SD}) 2.55$  (range 0–33,  $n = 368$ ) in 2000–2007 to  $0.198 \pm 1.43$  (range 0–16,  $n = 257$ ) in 2018. Infested *Myrmica* nests held more *M. alcon* X caterpillars and pupae in 2000–2007 (mean  $8.2 \pm \text{SD } 8.53$ , median: 5,  $n = 18$ ) than in 2018 ( $4.6 \pm 5.45$ ,  $n = 11$ ), but this difference did not quite reach statistical significance (median test,  $\chi^2 = 3.13$ ,  $P = 0.077$ ).

#### Host ant specificity

Extended Fisher's exact tests showed no evidence of host ant specificity in infection rates at any site in either period, although that for Bükkszentkereszt for 2018 was close to statistical significance ( $P = 0.063$ , Table 3). Randomisations of ant nests between species by MacSamp also did not show any specificity of numbers of *M. alcon* X caterpillars and pupae to ant species (Table 3). Taken together, these results are consistent with our previous finding of no heterogeneity in host ant use at these sites (Tartally *et al.*, 2008).

#### Discussion

Our study provides two key findings. First, several results showed that the *Myrmica* ant community changed slightly between 2000–2007 and 2018. Shannon diversity, infestation rate, and the mean number of caterpillars and pupae per nest decreased despite the increasing relative abundance of host ant species. Second, the changes in relative abundance and species composition differed amongst the sites, for example, MCSI decreased at three sites and increased only at Lófő-tisztás (Table 3). This is the only site where management was restarted after two decades of abandonment (Table 1).

There are some notable changes in the structure of the *Myrmica* communities, such as variation in dominance, species loss or substitution. It is possible that some of these changes are driven by climate changes as well as changes in management, although the pattern seen here is not consistent, as both warm-loving (*M. schencki*) and more cold-tolerant (*M. ruginodis*) species (Elmes *et al.*, 1998) were only recorded in the second survey at some sites. The apparent absence of *M. lona* in 2018 is intriguing, as the quantitative differences used to distinguish this species from *M. sabuleti* (Seifert, 2000) were clearly present in 2000–2007, but could not be found in 2018. Whether this represents true species loss or a temporal change in a plastic phenotype remains to be clarified. The continued or increasing dominance of *M. scabrinodis* on three of the sites is noteworthy, but is difficult to interpret as there seem to be at least two divergent lineages within this species that differ in their habitat requirements (Ebsen *et al.*, 2019) and may also differ in their suitability as hosts of *Maculinea* butterflies (Tartally *et al.*, 2019).

In general, results from our sites with little or no management suggest negative trends in *Maculinea* populations in the last decade. In Bükkszentkereszt and Kecskeláb-rét, the MCSI was an order of magnitude lower two decades after abandonment

than only a decade after. In Nagy-mező, MCSI was already low in 2000–2007 (only two infested ant nests) and it decreased to zero (no infested nests) by 2018. This result suggests that low-intensity horse-grazing was not successful in maintaining host ant and *Maculinea* populations, and that 9 years after abandonment the *Maculinea* population had likely gone locally extinct. This was surprising because a conspicuously abundant flight of *M.alcon X* was observed in the summer of 2012 (Table 1), in a year when this site was ravaged by a wildfire in early spring. This observation suggested that fires in the early spring do not damage overwintering, still inactive populations of *Myrmica* and *Maculinea* species, a finding also reported by Nowicki *et al.* (2009). Although *Myrmica* ants are sensitive to changes in the cover of vegetation (Grill *et al.*, 2008), fire can even have beneficial effects on *Myrmica* and indirectly on *Maculinea* species by removing dead plant material and changing the microstructure of the vegetation. The potential use of fire as a management tool (prescribed burning) should be explored in further studies.

There was no evidence for host ant specificity for *M.alcon X* on the four sites examined, despite reasonable sample sizes of *Myrmica* nests of different species, which often limits the ability to detect heterogeneity in host use (Tartally *et al.*, 2019). This may also reflect the particular *Myrmica* community composition on these sites, as although there were large changes in the relative abundance of different species, the proportion of species that had been recorded as local hosts of *M.alcon X* remained high. Changes in *Myrmica* ant community are likely to have an even larger effect if the proportion of host species changes, which will be more likely for sites where there is more host any specificity, as is generally the case for other *Maculinea* such as *M.arion*, *M.nausithous* and even *M.alcon H* (Tartally *et al.*, 2019). There was also no clear relationship between *Myrmica* nest density on the four sites that we examined and the presence and number of *M.alcon X* caterpillars and pupae. This suggests that it is not simply the density of potential host ants that is important for the maintenance of *M.alcon X* populations, but other factors associated with management. These could be related to *Myrmica* diversity per se, perhaps through the selection pressures imposed by the geographic mosaic of coevolution (Thompson, 1999; Tartally *et al.*, 2019), or to other factors that we did not measure, such as the size of host ant nests or changes in host plant populations (Mouquet *et al.*, 2005).

In summary, our results suggest that abandonment, that is, the termination of management, leads to changes that are unfavourable to the xerophilous form of *M.alcon* populations in the Bükk Mountains. The same trend was clear in the nearby Aggtelek region, but was successfully reversed by resuming management (Árnyas *et al.*, 2006). It should be noted that caution is warranted in the interpretation of our results because the management history of the study sites was sketchy and more details on, for example, livestock grazing pressure or frequency of mowing could have further refined the trends found. However, such detailed information was not available for the sites. Moreover, caution is also warranted because data referred to as '2000–2007' were collected during eight summers (Tartally *et al.*, 2008), whereas data from 2018 were collected in a single summer. The size of *Maculinea* populations tends to be quite variable between years, often showing something approaching cyclic changes in the

adult population (see e.g. Nowicki *et al.*, 2009), so the decrease in the number of larvae in one single monitoring event (2018) could be due to a low adult population the year before that is not linked to environmental causes. As no information was available about the adult and/or egg densities in the previous years, we cannot conclude that the background of this decrease of *M.alcon* larvae by 2018 is entirely the change of the *Myrmica* community. All of these uncertainties emphasise the importance of more detailed and long-term monitoring of *Maculinea* populations in the future.

The patterns that we observed suggest that management of mountain grasslands (former hay meadows) is crucial for maintaining rich and diverse communities of *Myrmica* ants, which may also be crucial for *M.alcon X* populations. The observed changes in *Myrmica* communities combined with what is known of the histories of the sites suggest that the best way to manage these habitats is a combination of regular low-intensity mowing and low-intensity grazing. Such management has been started recently at Lófő-tisztás, and changes in the ant community and *Maculinea* numbers attest that such management can lead to favourable changes in the habitat. Further monitoring is necessary to establish the exact mechanisms of these favourable changes. It is, however, important to note that management proven successful in one type of habitat or population may not necessarily be successful for other habitats or populations. For example, *Maculinea* populations are also affected by large-scale factors such as latitude, altitude and climate (Thomas *et al.*, 1998b).

Our study shows that it is important to conduct long-time monitoring of the effects of site management on host ant use of *Maculinea* butterflies. Because land abandonment occurs on large spatial scales in central and eastern Europe, such monitoring should be extended to as many *Maculinea* populations as possible to compare these effects and to design conservation actions for *Maculinea* butterflies and other species of mountain hay meadows.

## Conclusion

We conclude that abandonment, that is, termination of regular management, of mountain hay meadows presents significant risks to the persistence of *Maculinea* populations. Re-starting management by low-intensity mowing and low-intensity grazing can increase the suitability of habitats to *Maculinea* butterflies and their host ants. Long-time monitoring of the effects of site management is important for the successful protection of *Maculinea* populations.

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